Interactions of invertebrates, micro-organisms and tree roots in nitrogen and mineral element fluxes in deciduous woodland soils

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SUMMARY

1 There is increasing evidence that soil invertebrates have quantitatively important roles in the nutrient flux pathways of forest soils, particularly in the mobilization and mineralization of nitrogen through direct and indirect effects.

2 The direct effects are simple transfers of elements through food webs which can be estimated, with variable difficulty, from population data. Indirect effects involve feedbacks on microbial populations and activities, and are poorly quantified.

3 Short-term, indirect effects of macrofaunal feeding activities result in enhanced nitrification and ammonification in forest litters and soil organic matter.

4 A model is proposed which attempts to quantify and predict nitrogen mineralization through interactions of micro-organisms and invertebrate saprotrophs.

5 Field experiments using microcosms and small lysimeters, both with integrated tree root systems, demonstrate further interactive effects of animals on nitrogen fluxes during wetting and drying cycles. There are also indications that animals may facilitate mineral uptake by roots.

INTRODUCTION

The organic nitrogen pools within litter and soil organic matter (SOM) are the main sources of nitrogen which sustain primary productivity in natural forests or unfertilized forestry systems (Aber & Melillo 1980). The timing and extent of the mobilization of this nitrogen (net mineralization) in relation to plant requirements is a key process governing the successional development and the climax productivity of the system (Clark & Rosswall 1981; Melillo & Gosz 1983). In addition, the response of the nitrogen cycle to disturbance, including various aspects of management practices, is to a large extent determined by the capacity of the litter and soil to retain or release nitrogen (Miller 1979; Swank & Waide 1980; Heal, Swift & Anderson 1982; Vitousek *et al.* 1982).

Net mineralization represents the balance between the key processes of gross nitrogen mobilization (predominantly as ammonium but also as simple organic forms, such as amino acids, which are available for root mycorrhizal uptake) and

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immobilization in decomposer biomass or through absorption of ammonium onto soil (Nömmik 1981; Kudeyarov 1981). The availability of nitrogen for root uptake may also be influenced by the balance between rates of nitrification and losses from the system as a consequence of denitrification and leaching. Most terrestrial ecosystems are highly conservative of nitrogen–especially forests with acid, organic soils–and losses of nitrogen are small compared with the internal fluxes. Therefore, the net mineralized nitrogen potentially available to higher plants represents the fraction of gross nitrogen mineralization which exceeds microbial demand (Runge 1971) and ammonium fixation. These latter two processes will be inversely related to the availability of nitrogen (and phosphorus) in the system as a consequence of nitrogen limitations on decomposition proceses and the accumulation of large SOM pools. The processes which govern the relationships between net mineralization, gross mineralization and the nitrogen pool in SOM are therefore critical parameters of ecosystem functioning in forest soils.

At a systems level the operation of these processes and their correlation with other ecological variables and physical environmental parameters are increasingly well documented, but there has been little explicit recognition of the complexity of mechanisms which regulate nutrient fluxes between litter, soil and plant roots (Frizzel & van Veen 1982), particularly in forest ecosystems. Specifically, the role of soil fauna in nitrogen flux pathways has received scant attention and the rationale for nitrogen mineralization processes is usually based almost entirely on microbial processes. However, there is mounting evidence that soil animals significantly disrupt the time course of microbial processes and enhance the turnover of microbial populations through direct and indirect effects (Anderson & Ineson 1984).

DIRECT AND INDIRECT EFFECTS OF ANIMALS ON NITROGEN MINERALIZATION

The direct effects of invertebrates on fungi and bacteria involve the release of elements through trophic transfers and population processes: feeding, excretion, and the turnover of secondary production. The indirect effects include feedbacks to lower trophic levels: for example, the effects of litter comminution and grazing on microbial activity and the functional organization of bacterial and fungal communities (Anderson & Ineson 1983), as well as predator/prey interactions. The central problem is quantifying both the direct and indirect effects of soil invertebrates on microbial processes in the context of ecosystem-level nutrient fluxes.

The direct contribution of invertebrates to nutrient fluxes can be quantified using a budgetary approach where sufficient information on the biology and population ecology of the decomposer community is available. Estimates of nitrogen turnover by invertebrate populations have been recently reviewed by Anderson, Coleman & Cole (1981), Seastedt (1984) and Anderson & Ineson (1984). Results from studies in a wide range of forest types suggest that in temperate forest soils with a mean annual invertebrate biomass of about 5–10 g (dry wt) m^{-2} (i.e. 20–30 g fresh wt) the annual turnover of nitrogen and other minerals by fauna can equal or

exceed inputs to the decomposer system. In base-rich, deciduous woodland soils with an earthworm biomass of 50–100 g (fresh wt) m^{-2}, or even more (Satchell 1983), the annual nitrogen flux through the worm population may be several times the 30–70 kg ha^{-1} $year^{-1}$ contained in leaf fall (Satchell 1963). At the other extreme, in an acid pine forest soil in Sweden, Persson (1983) has estimated that the fauna, with a mean annual biomass of only 1–7 g (dry wt) m^{-2} (in comparison with 120 g m^{-2} fungi and 39 g m^{-2} bacteria) contributed between 10 and 49% of annual nitrogen mineralization, of which 70% was excretion by bacterivores and fungivores.

We have demonstrated significant indirect effects of macrofauna on nitrogen release from forest litter and SOM in the laboratory (Anderson, Ineson & Huish 1983; Anderson & Ineson 1984). For example, millipedes feeding on oak leaf litter with a C:N ratio of 100 over a period of 15 weeks effected the release of 9·7% of the nitrogen capital in the leaves compared with 3% from controls without animals. Experiments using ^{15}N labelled millipedes and leaf litter showed that only 7·5% of the mobilized nitrogen was attributable to the excretion of the animals (Anderson & Ineson 1984). The indirect nature of these effects, through interactions with bacteria and fungi in the litter, is also demonstrated by the fact that the nitrogen release does not occur in a step-wise manner when the animals are added or removed from the experimental system, but takes several weeks to build up or decline (Anderson & Ineson 1984). The enhancement of nitrification by animal feeding activities is also indicative of indirect effects on nitrogen mineralization (since invertebrates do not excrete nitrate).

We review here a series of experiments which attempt to validate and quantify these effects on nitrogen mineralization both in the laboratory and in the field. The emphasis in this paper is on the general approach since detailed discussion of results cannot be made here. We firstly consider the variables influencing animal-mediated nutrient fluxes and propose a series of models quantifying animal and microbial effects. We then consider the dynamics of nitrogen fluxes in field experiments with tree root systems, using microcosms and lysimeters to demonstrate animal effects at the ecosystem level.

PROCESS VARIABLES OF ANIMAL-MEDIATED MINERAL NITROGEN FLUXES

The process variables determining nutrient flux rates can be considered within a framework of resource type, the qualitative and quantitative characteristics of the organism community and physical environmental parameters (Swift, Heal & Anderson 1979).

Resource type

As a first step towards quantifying indirect animal effects we added millipedes, *Glomeris marginata*, to a range of leaf litter (L), fermentation layer (F) and humus

TABLE 1. Effects of feeding by *Glomeris* on nitrogen leaching from different organic soil layers of three oak woodlands in Devon

Soil layer	Stoke Wood		Perridge Wood		Brook Wood	
	Control	Animals	Control	Animals	Control	Animals
Litter layer (L)						
NH_4–N	18·8 ± 5·7***	183·3± 10·3	23·7 ± 3·2*	251·1 ± 81·3	38·0 ± 19·5***	591·3 ± 69·6
NO_3–N	1·6 ± 0·4*	3·5 ± 0·7	1·7 ± 0·4	3·7 ± 1·0	1·1 ± 0·5	5·5 ± 1·5
Fermentation layer (F)						
NH_4–N	37·2 ± 8·4***	259·3 ± 24·3	217·9 ± 50·7**	513·9 ± 48·2	716·4 ± 194·4*	1333·5 ± 90·0
NO_3–N	46·8 ± 28·2	16·4 ± 4·4	12·7 ± 9·5	4·4 ± 0·6	17·6 ± 5·2	61·2 ± 31·6
Humus layer (H)						
NH_4–N	15·3 ± 2·1*	188·7 ± 50·7	131·0 ± 7·2***	214·7 ± 4·1	107·1 ± 6·5**	188·8 ± 17·5
NO_3–N	116·1 ± 0·5***	384·5 ± 50·3	2·3 ± 0·3	2·1 ± 0·2	13·9 ± 3·2	9·5 ± 2·0

Results are expressed as mean cumulative concentrations of mineral nitrogen (μg g dry litter^{-1} ± SE; n = 3) mobilized as ammonium-N or nitrate-N over a period of 5 weeks after the addition of four animals to the animal treatments. Values for controls (without animals) are shown for the same period of time. The experiments were incubated at 15°C. Significant differences between animal treatments and controls are shown as *** ($P < 0·001$), ** ($0·001 < P < 0·01$) and * ($0·01 < P < 0·05$) (Anderson & Ineson 1984).

materials (H) to investigate the effects of resource type on animal mediated nitrogen fluxes (Anderson & Ineson 1984). Samples were collected from three oak–beech woodlands (*Quercus robur* with small *Fagus sylvatica*) in May 1982. Materials were air dried, lightly crushed and then sieved to prepare a 5–20 mm fraction of litter and a 1–3 mm fraction of humus. Aliquots of 1·5 g litter and 3 g humus were added to microcosm chambers (Anderson & Ineson 1982), rehydrated with distilled water and inoculated with a suspension of fresh litter or humus. The chambers were then incubated at 15°C for 5 weeks before animals, four *Glomeris marginata* weighing 0·4–0·5 g (fresh wt), were added to chambers designated as animal treatments. The chambers were irrigated every 7 days with distilled water and the leachates analysed for mineral-N as nitrate and ammonium.

The results summarized in Table 1 show that the animals significantly enhanced ammonification in all sites and resource types, though the magnitude of the response is variable both within and between sites. The effects on nitrification are more variable and insignificant in most cases except for the Stoke Woods humus. This H-layer material showed a characteristic dominance of nitrate-N in controls which was maintained in animal treatments although the effects of animals were larger on the ammonium-N fluxes (enhanced twelve times) than on the nitrate-N fluxes (enhanced three times).

Losses of ammonium through nitrification complicate interpretation and therefore we will briefly consider these results in terms of total mineral nitrogen. Animal feeding activities increased mineral-N losses from the L-layer materials by ten to fifteen times control levels but only by two to three times in the F-layers and by even less (1·6 times) in the H-layer materials from Perridge and Brook Wood. The humus from Stoke Woods showed total mineral-N losses over four times control levels in animal treatments.

We interpret these differences in control and animal-mediated fluxes, both within and between sites, in terms of the reduced availability of nitrogen to microorganisms with the advancing stages of decomposition (Hayes & Swift 1978). Microbial immobilization of nitrogen is generally considered characteristic of the early stages of decomposition, especially in low quality resources, but this is disrupted by the feeding activities of animals in the litter-layers. The animals may therefore reduce nitrogen transfers to the more intractable humus pool and mobilize nitrogen in close proximity to the root/mycorrhizal network which is characteristically located at the litter/humus interface in these woodlands. In the Stoke Woods site, the higher availability of nitrogen in the humus material is reflected by the larger animal effects on nitrogen fluxes than in humus from the other two sites. The level of nitrification which is found in this site is not reflected by differences between Stoke Woods and similar woodlands in the area in terms of soil pH, total nitrogen content of the humus (ranging from 1·3 to 1·5%), parent minerals or vegetation type (Anderson *et al.* 1985). The scale of animal effects on humus nitrogen mineralization does, however, differentiate this site from the others and we are investigating the use of this response as an index of nitrogen availability.

Organisms

Our approach in these studies has been to try and find a level at which to compartmentalize animal–microbial interactions of individual species or organism groups. As a first step towards this abstraction, a necessary requisite for a simple model, we have investigated the conditioning of litter by different species of fungi (Anderson & Ineson 1984) and the activities of different faunal groups in mobilizing nitrogen (Anderson, Ineson & Huish 1983; Anderson & Ineson 1984).

Freshly fallen oak litter was collected from litter traps, autoclaved and then inoculated separately with six species of fungi, including four basidiomycetes, and incubated for several weeks before millipedes were added. The results showed that the mineral nitrogen mobilized by the animals (N_a) was related to the nitrogen mineralization by the controls (N_c) according to the function

$$N_a = 9{\cdot}3\ N_c - 124{\cdot}2\ (r = 0{\cdot}74, f = 16, P < 0{\cdot}001)$$

where N_a and N_c are in μg g leaves^{-1}. This function is specific to this resource type but suggests that effects of the animals transcend the taxonomic identity of the fungus. We have no indications from all the other experiments we have carried out using mixed inocula from homogenized litter to doubt the general validity of these conclusions, but the effects of fungal species conditioning leaf litter on animal feeding activities requires further study.

A similar approach was used to investigate the roles of different invertebrate groups on nitrogen mineralization (Anderson, Ineson & Huish 1983). Enchytraeids, collembola, earthworms and a range of millipede groups were added to oak leaf litter in numbers chosen to demonstrate the maximum likely effects which might occur within aggregated field populations. It was found that the overall effect of animal groups on ammonium-N release in mg g litter^{-1} over a 6-week period related linearly to biomass (B) acording to the function

$$N = 1{\cdot}6\ B\ (r = 0{\cdot}86, f = 22, P < 0{\cdot}001)$$

where B is expressed in mg fresh wt of animal. This relationship, although again resource specific, suggests that the taxonomic identity of the animals is not as significant a variable as their biomass and activity. We acknowledge that these relationships need further investigation but in the light of these results we have chosen the millipede *Glomeris marginata* as a tool for modelling the indirect effect of litter-feeding animals on nitrogen mineralization.

ANREG: nitrogen mineralization as a function of temperature and animal biomass

A regression model has been developed quantifying and predicting the effects of soil and litter macrofauna on nitrogen mineralization. Leaf litter (F) and humus (H) materials collected from three oak–beech woodlands in Devon (Perridge, Stoke and Hillersdon Woods) were incubated in laboratory microcosms, with and without animals, at three temperatures (5, 10 and 15°C) which are representative of the

mean monthly F–H layer temperatures recorded in the field (Fig. 2c) when soil fauna are active. At higher temperatures moisture usually limits animals and microbial activities, especially in the litter layers, as considered below. Three animal treatments were used which were considered to be high but not unrealistic of biomass (fresh wt) to resource (dry wt) quotients found in the field: 0·1, 0·2 and 0·3 g animals g litter $^{-1}$ and 0·03, 0·07 and 0·1 g animals g humus $^{-1}$. The methods are essentially the same as those outlined above for resource types.

Nitrogen mineralized as nitrate-N or ammonium-N (N_A in μg N g resource $^{-1}$) from animal treatments (B as defined above and including controls as zero biomass) and at the three temperatures (T°C) was monitored weekly over a period of 8 weeks after the animals were added. Periods of 5, 6 and 7 weeks were analysed using the following multiple regression (which we term ANREG):

$$N_A = a + bT + cB + dBT$$

Nitrogen mineralized over 6 weeks showed the best fit to ANREG for all sites, resource types and forms of mineral nitrogen. For the purposes of the present discussion we will only consider ANREG functions for total nitrogen in Perridge and Stoke Woods to illustrate the model. Details are published by Anderson *et al.* (1985).

Equations for the litter are (± SE for partial regression coefficients):

Perridge: $\mathbf{N_A = 14{\cdot}3(\pm 38{\cdot}3) + 31{\cdot}6(\pm 3{\cdot}5)T - 135(\pm 102)B + 47{\cdot}8(\pm 9{\cdot}5)\ BT}$
$R^2\% = 94{\cdot}2,\ F_{3,32} = 191{\cdot}7,\ P < 0{\cdot}01$

Stoke: $\mathbf{N_A = 171(\pm 108{\cdot}7) + 41{\cdot}4(\pm 10{\cdot}1)T + 504(\pm 191)B + 74{\cdot}6(\pm 26{\cdot}9)\ BT}$
$R^2\% = 87{\cdot}6,\ F_{3,32} = 158{\cdot}7,\ P<0{\cdot}01$

and for the humus:

Perridge: $\mathbf{N_A = 26{\cdot}5(\pm 3{\cdot}8) + 4{\cdot}2(\pm 0{\cdot}4)T + 15{\cdot}0(\pm 10{\cdot}3)B - 0{\cdot}07(\pm 0{\cdot}9)\ BT}$
$R^2\% = 92{\cdot}1,\ F_{3,32} = 158{\cdot}7,\ P < 0{\cdot}01$

Stoke: $\mathbf{N_A = 165(\pm 28) + 8{\cdot}0(\pm 2{\cdot}6)T - 115(\pm 74{\cdot}7)B + 21{\cdot}7(\pm 6{\cdot}9)\ BT}$
$R^2\% = 75{\cdot}6,\ F_{3,32} = 37{\cdot}1,\ P<0{\cdot}01$

The resource quality differences between the litter and humus materials are reflected in the partial regression coefficients for temperature and biomass which vary, significantly in most cases, between sites as well as resources.

There is generally poor agreement between levels of nitrogen mineralization predicted by ANREG for Stoke and Perridge Woods and the values shown in Table 1. Specifically, that ANREG tends to overestimate nitrogen mineralization from the controls (run with zero biomass) and underestimate animal effects (the sum of B and BT functions). These differences will not be discussed in detail because their magnitude and significance is less important here than the value emphasizing the uncontrolled parameters which may preclude comparisons of these experiments.

It can be seen from Table 1 that surface leaf litter (L) showed low levels of nitrogen mineralization by controls but this was enhanced ten to fifteen times by

the animals. As decomposition progresses and the material becomes classifiable as F-layer, microbial mineralization of nitrogen increases but the scale of the indirect animal effects generally decreases. Finally, in the H-layers of these woodland types the nitrogen in humus usually becomes more intractable to animal and microbial activities (Stoke Woods is the main exception to this pattern which we have encountered). The partitioning of these materials according to time–depth relationships is therefore critical for experimental work since the combination of resource types with different net nitrogen mineralization potentials will influence the interpretation of animal and microbial effects. But the relationship between the decomposition time-series and soil horizons is open to question for the reasons outlined by Swift *et al.* (1979) in formulating a cascade model of decomposition processes. Particulate materials and soluble organic matter will be separated from the parent litter-class by comminution and leaching and move down the profile to depths determined by the resource characteristics, animal activities, soil structure and processes of transport. These cascade processes have not been quantified in any soils but the overall magnitude and their seasonal pattern must be a fundamental characteristic of different forest types.

ANREG was not determined for L-layer materials because previous experiments had led us to believe that the seasonal change in net nitrogen mineralization potential of this litter was more variable than materials from the F- and H-layers. We now feel that small differences in the combination of these resources, whether a feature of sampling procedure or through seasonal variations in animal activities, can influence the outcome and interpretation of these experiments. ANREG, however, offers a means of quantifying these effects including, for the first time, a measure of the indirect effects of macrofauna on nitrogen mineralization. Thus, on the basis of the present model we predict that litter-feeding animals will significantly contribute to nitrogen mineralization in forest soils where the biomass/resource quotient B is greater than 0·1 (Anderson *et al.* 1985). Under these conditions net nitrogen mineralization may be occurring in the field where laboratory incubations, without animals, show nitrogen immobilization. As an intermediate step towards investigating these effects in the field, and for understanding the functioning of complex systems, subject to extremes of temperature and precipitation, we established tree roots in the microcosms to investigate animal and root interactions in mineral element fluxes.

ANIMAL AND ROOT EFFECTS IN MICROCOSMS

The standard experimental chambers used in laboratory studies (Anderson & Ineson 1982) were set up in the Perridge field site with root systems established in a small quantity of F-layer material beneath the inner chambers containing the experimental material. Oak tree root systems, with extensive ectomycorrhizal development, were carefully dissected from the surface humus layers and 10–15 cm of the attached root network were introduced through a slit in the outer chambers which were then sealed with silicone grease. The chambers were estab-

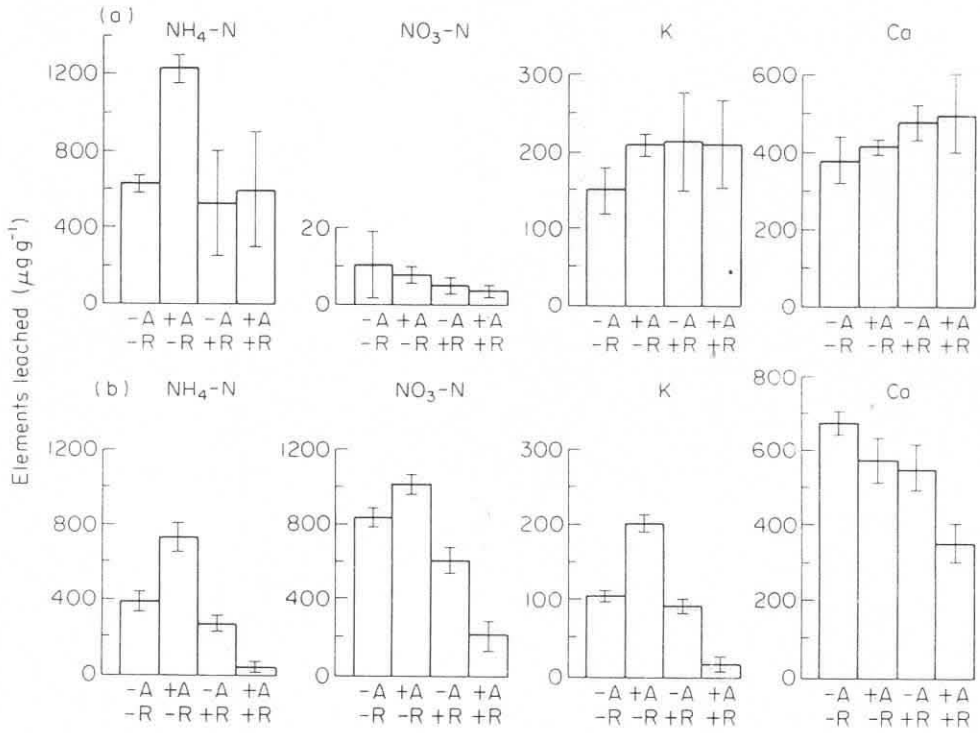

FIG. 1. Nitrogen, potassium and calcium losses from leaf litter and humus in field experiments using microcosms with and without animals and tree roots. Values are cumulative ($\mu g\ g^{-1}$ material ±SE) over 6 weeks for the Perridge F-layer material (a) and over 4 weeks for the Stoke Woods H layer materials (b) in experiments carried out sequentially using the same undisturbed rooting systems (for further details see text).

lished in early May using Perridge F-layer material and the animals introduced 8 weeks later when the root/mycorrhizal systems appeared healthy and showed growth. Leachate concentrations of mineral nitrogen, potassium and calcium were monitored for 6 weeks. In mid-August the inner chambers were then replaced with units containing humus material from Stoke Woods which had been maintained in the laboratory at 15°C, with and without animals, for several weeks before being set up in the field microcosms. The results of these two experiments are shown in Fig. 1.

Losses of nitrogen as nitrate were negligible from all the Perridge F-layer treatments (Fig. 1a) while treatments with animals showed nearly twice the cumulative ammonium-N losses of treatments without animals. The treatments with roots, but without animals, showed very similar ammonium-N losses to the non-rooted treatments suggesting negligible uptake of mineral nitrogen by the roots. But the differences between nitrogen losses from the rooted systems with and without animals suggest that there has been a transfer of all the animal-mobilized nitrogen to the roots. Potassium losses show a 30% increase attributable to animals

in the non-rooted systems but both of the treatments with roots showed similar increases even when animals were absent. Calcium losses are also higher in the rooted treatments, and there are no significant animal effects.

The apparent facilitation by animals of ammonium uptake by roots is a consistent feature of similar experiments using root implants in microcosms but one which we are unable to explain at the present time. The increased potassium and calcium losses from the rooted systems are more readily attributable to root exudates. Smith (1976) showed that potassium and calcium were the dominant cations in exudates from unsuberized roots of *Betula*, *Fagus* and *Acer* species. Cumulative values over 14 days ranging from 4·2 to 12·8 μg K mg root^{-1} and 2·0 to 4·8 μg Ca mg root^{-1} are compatible with the scale of effects noted here.

The dynamics of nitrogen, potassium and calcium fluxes are totally different when the inner chambers containing Perridge litter were replaced with the Stoke Woods humus layer material (Fig. 1b). It should be noted that the same rooting systems were involved and the roots were not disturbed by the sample changes. There were also no major changes in soil temperatures over the period of the two experiments. The transition in the dynamics of the system was abrupt when the Stoke Woods material was added to the systems and the predominantly ammonium-N fluxes of the F-layer material was replaced with the strongly nitrifying H-layer material. Approximately 60% of the mineral nitrogen released from the humus was in the form of nitrate.

Interpretation of the ammonium-N fluxes, with and without animals, is complicated by losses of ammonium through nitrification. In terms of total nitrogen, treatments without animals and roots showed losses of 1·2 mg compared with 1·8 mg in the presence of animals; an increase of 50% net mineralization through animal effects. Total nitrogen losses from the rooted and non-rooted treatments, without animals, were not significantly different (1·1 mg g^{-1} and 1·2 mg g^{-1} respectively). Only 0·2 mg was recovered from the rooted treatments with animals. This suggests that 80% of the animal-mobilized nitrogen was taken up by the roots, half in the form of nitrate, and that animals apparently facilitated this uptake of mineral nitrogen as in the earlier experiment.

There are reports that nitrate inhibits mycorrhizal infection in natural soils, though attempts to differentiate betwen the effects of pH and other associated soil parameters have not been very satisfactory (Alexander 1983). Cole (1981) assumes ammonium to be the preferred form of nitrogen by forest trees since it is the form the mycorrhizas are most likely to encounter. If this is correct then naturally-occurring mycorrhizas might not be expected to take up nitrate readily. This is suggested by the work of Bledsoe (1976) on *Pseudotsuga* grown in culture, and excised beech mycorrhizas also show negligible uptake of nitrate from solution (Smith 1972). Field evidence is generally lacking though Haines (1977) has shown a low uptake of nitrate applied to the soil of a mixed pine–oak woodland. However, it is increasingly recognized that nitrate may represent a significant component of mineral nitrogen in forest soils and that the correlations between nitrification and soil pH is a dogma which does not survive close examination (Robertson 1982).

The high nitrification rate of the Stoke Woods humus material is an example of this phenomenon and the facultative ability of mycorrhizas to take up nitrate, as we believe we have demonstrated, is entirely consistent with nitrogen conservation in forest soils which show periodic pulses of nitrification.

The utilization of nitrate by mycorrhizas has important implications for the mineral cation nutrition of plants since the nitrate can act as a 'carrier' (Bledsoe 1976; Raven & Smith 1976; Kirkby 1981). Pulses of nitrification in these forest soils may therefore facilitate cation uptake, and the results presented in Fig. 1b support this hypothesis. In contrast to the negligible root effects on potassium and calcium losses from the ammonifying F-layer material, comparison of the animal treatments, with and without roots, in the H-layer material suggests that the roots have taken up 84% of the potassium and 34% of the calcium. We are unable to account for the reduction of potassium and calcium losses from the rooted systems except by root uptake. We have previously shown (Anderson & Ineson 1984) that it is possible to predict calcium (in μg g humus^{-1}) mobilization by animal grazing in humus from the relative nitrification index (RNI) defined as the nitrate concentration divided by total mineral nitrogen concentration, according to the function

$$Ca = 11{\cdot}5\ RNI + 3{\cdot}6 \qquad (r = 0{\cdot}90,\ df = 19,\ P<0{\cdot}001)$$

The mechanism for this effect is believed to be the mobilization of calcium from exchange sites on SOM by hydrogen ions released through enhanced nitrification. Thus, in the absence of root uptake, and in a strongly nitrifying humus, the two animal treatments should show enhanced calcium (and potassium) losses. Clearly we must consider the ionic balances of these systems in detail before reaching any firm conclusions, but we discount denitrification as balancing these effects, though it has yet to be measured for these systems, since it would have to occur differentially in the treatments with roots and animals.

ANIMAL AND ROOT EFFECTS IN FIELD LYSIMETERS

Twelve 0·5 × 0·5 m by 30 cm deep replicate lysimeters were established in October 1982 in the Perridge oak woodland site. The lysimeters were set up as large analogues of the field microcosms with mean litter and SOM standing crops for the site. Large samples of bulked materials were air dried to extract the fauna and aliquots, equivalent to 251 g dry wt m^{-2} humus, were rehydrated before adding to the lysimeters. Extensive tree root systems were then introduced through ports in the sides of the trays, which were sealed around the roots, and all twelve units were covered with 1·5 mm mesh netting to exclude macrofauna. Periodic checks were made of all treatments up to April 1983, and thereafter of controls, to locate and remove small earthworms which entered the lysimeters. Leaf litter fall, precipitation and soil temperatures were monitored on the site and mineral concentrations in throughfall and lysimeter leachates measured every week.

Interpretation of results is complicated since the detection of differences between treatments depends upon rainfall; but the intensity and duration of rain, and

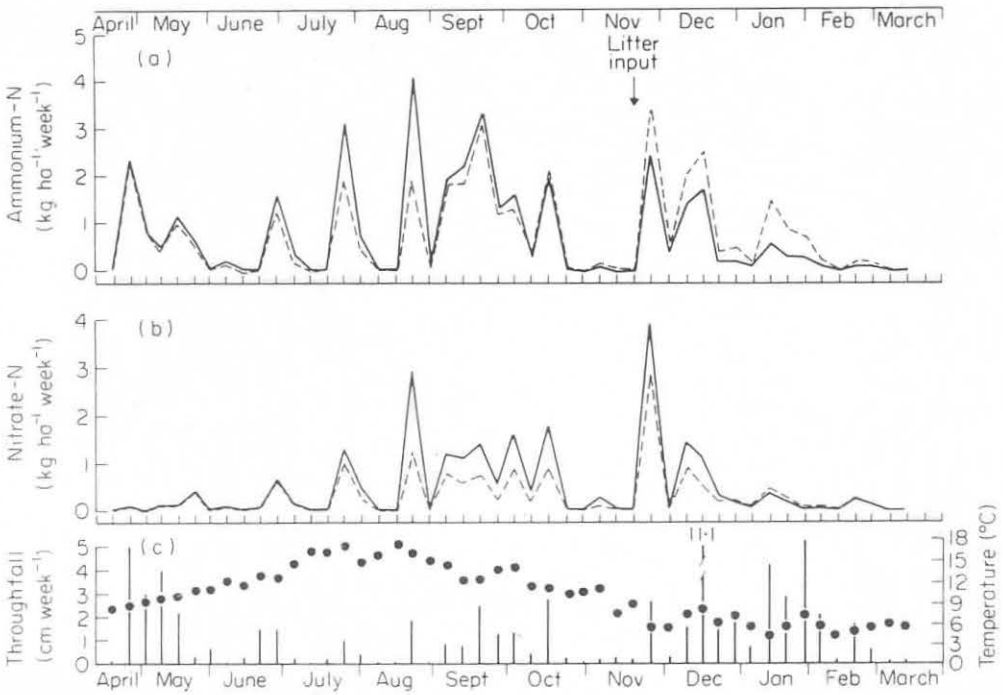

FIG. 2. Nitrogen losses as (a) ammonium-N and (b) nitrate-N from small lysimeters in an oak woodland with (——) and without (– – –) litter-feeding animals. The animals were added in April to established systems; leaf litter aliquots were added in November as indicated. Throughfall volumes (bars) and mean humus temperatures (●) measured at 5 cm below the surface litter (c).

the different effects of roots when the trees are bare or in leaf, affect leachate volumes and concentrations of elements. We will therefore briefly consider nitrate and ammonium fluxes in the treatments with and without animals, but without roots (Fig. 2).

In April 1983, 3·5 g (fresh wt) of millipedes, woodlice and earthworms (equivalent to 14 g m^{-2}) were added to three lysimeters with roots and three lysimeters without roots. Hereafter the discussions of animal treatments refer to these six units; the development of micro- and mesofauna populations following rewetting was assumed to be similar in all treatments.

No significant animal effects were recorded for 14 weeks after the addition of animals, but after that ammonium and nitrate concentrations in leachates increased relative to treatments without animals and were significantly higher in most samples taken from the beginning of July to mid-November. During this period the treatments without animals lost the equivalent (cumulative mean ± SE) of 17·3 ± 0·5 kg ha^{-1} ammonium-N and 8·6 ± 0·4 kg ha^{-1} nitrate-N, compared with 19·5 ± 0·6 kg ha^{-1} ammonium-N and 15·4 ± 1·5 kg ha^{-1} nitrate-N from animal treatments, a significant difference ($P < 0·05$) of 8·9 kg ha^{-1} total-N attributable to the presence of litter-feeding animals.

During July and August there were three periods of high soil temperatures and no rainfall which were followed by large nitrogen losses from the lysimeters when the soil was rewetted: up to 2·2 kg ha^{-} ammonium-N and 1·6 kg ha^{-} nitrate-N greater from animal treatments than controls during the course of 1 week.

The enhancement of nitrogen mineralization in the controls without animals can be interpreted in terms of wetting and drying effects on soils, though the mechanisms are not well understood (Van Veen, Ladd & Frissel 1984). The mineral nitrogen flush is thought to be the consequence of increased microbial activity resulting from the mobilization of organic substrates from SOM or the lysis of microbial biomass (Jager & Bruins 1975; McGill *et al.* 1981; Jenkinson & Ladd 1981). There are few experiments which can be used to interpret the animal effects in our systems. Witkamp & Frank (1970) observed that upon rewetting leaf litter the animals (millipedes) continued feeding at the same rate as before drying but there was a characteristic flush of microbial activity followed by nutrient immobilization during the subsequent phase of microbial growth. It was concluded, in general terms, that nutrient mobilization through wetting and drying cycles would be more significant than animal effects at high temperatures although analysis was not carried out for nitrogen mineralization and immobilization in these experiments.

We have shown in the laboratory that the effects of animals on nitrogen mineralization do not occur in a step-wise manner when the animals are added, but take time to build up, and continue for a period of weeks after the animals are removed (Anderson & Ineson 1984). We have also shown (J. M. Anderson, unpubl.) that drying of litter or humus, followed by short wet periods of a week or so, is insufficient to elicit significant animal enhancement of nitrogen mineralization and the declining differences between animal treatments and controls, following each dry–wet cycle, are equivalent to removing the animals completely. thus, wetting and drying events inhibit continuing animal enhancement of nitrogen mineralization but enhance the differences between treatments through mobilization of some labile nitrogen pool formed by animal feeding activities, a phenomenon demonstrated by these field results.

Leaf litter (300 g m^{-2} and equivalent to inputs of 30 kg N ha^{-1} year^{-1}) was added to the lysimeters in late November and during the following week of heavy rain total inorganic nitrogen losses amounted to 9·6 ± 0·2 kg ha^{-1} from treatments without animals and 6·4 ± 0·2 kg ha^{-1} from treatments with animals (Fig. 2). Since this period was preceded by, and followed by, weeks without rainfall, interpretation of this effect is difficult but the significant difference ($P<0·01$) of 3·2 kg ha^{-1} week^{-1} between treatments is the reverse trend in terms of the faunal effects expected from the wetting and drying events considered above. This points to further interactive effects of animals in soil processes resulting in nitrogen conservation during the winter period. The total nitrogen losses from the rooted lysimeters were about 30% of those from the unrooted systems. Furthermore, nitrogen losses from treatments with roots and animals were generally 12–16% lower than from treatments containing roots alone. This represents a cumulative difference of approximately 3·5 kg N ha^{-1} year^{-1} and substantiates the animal enhancement of root effects demonstrated in the field microcosms.

CONCLUSIONS

We have demonstrated, under laboratory and field conditions, that litter-feeding animals can have significant qualitative and quantitative effects on nitrogen mineralization from leaf litter and SOM which can not be predicted from population data alone. Specifically, we have shown that the animals disrupt the time course of microbial mineralization processes, and net mineralization of nitrogen is enhanced at carbon:nitrogen ratios where microbial immobilization of nitrogen in SOM would be expected to occur (Sollins, Spycher & Glassman 1984). Preliminary attempts to quantify these effects using ANREG suggest that they may significantly contribute to nitrogen mineralization when the resource to biomass ratio (B) is greater than 0·1. In fact, the potential magnitude of the animal-mediated flux may be predictable from the maximum value of B attained in a forest soil since both animal biomass and litter decomposition rates are related functions of nitrogen (and phosphorus) availability.

Field lysimeters demonstrate significant animal effects on nitrogen mineralization at B-values initially established at 0·06 for litter and 0·03 for humus, assuming for the purposes of this discussion that all the animals were active in either resource. on this basis one would predict that the enhanced nitrogen mineralization resulted from their feeding activities in the litter. While these conclusions are speculative, the fact remains that the effects of the animals in the lysimeters occurred at a lower macrofauna biomass than is found in many temperate deciduous, and some coniferous, forest soils (Raw 1967; Satchell 1983).

The study of soil ecology is far from the point at which all the roles and activities of soil fauna, and their interactions with fungi and bacteria, have been identified, and there may be many soils and processes in which these interactions are functionally important. but the assumption of their insignificance must be from a position of insight and not from dogma. The methods and approaches outlined here are still at an early stage of developing a quantitative understanding of animal–microbial interactions but we do feel that the results, together with others presented in this volume, emphasize the need for more holistic approaches to investigating the mechanisms of soil processes.

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